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DIFFERENCES IN SUSCEPTIBILITY TO WHOLE-BODY GAMMA IRRADIATION IN THE LAYERS OF THE RETINA OF BUFO

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Differences in susceptibility to irradiation constitute a problem of biological significance heightened by the fact that they occur in comparable tissues in different groups of animals. We have found that in *Bufo* the cells destroyed under the conditions of our experiments include certain small nerve cells of the brain, of the olfactory membrane, and of the retina. This paper deals with the latter because it offers an especially instructive example of these principles.

Materials and Methods

Recently metamorphosed *Bufo boreas halophilus* with trunk lengths of 10 to 12 mm. were firmly held in the zone of maximum irradiation while being exposed to total-body irradiation by a cobalt^{60} source. The average dosage rate was 950 r/minute. Fixation was by Bouin's fluid. Serial paraffin sections were cut at a thickness of one to five microns. A thickness of three microns was the most suitable. Slides were left over-night in commercial H_2O_2 in order to expose the rods and cones by depigmenting the strands from the pigment layer.

Some material was stained with Delafield's hematoxylin and eosin, but by far more useful was Heidenhain's iron-alum hematoxylin, slides being left one-half hour in each of the two solutions. This stain proved especially valuable not only because of its sharpness but because it stained the pyknotic nuclei most intensely.

Results

Gamma irradiation of 10,000 r, 20,000 r, 30,000 r, and 60,000 r caused destruction to the nerve cells of the inner nuclear and ganglionic layers. The few nuclei surviving after a dose of 60,000 r may be largely identified as those of the fibers of Mueller and the amacrine cells. On the other hand, even 60,000 r within the twenty-four hour survival period of the toads caused no destruction to the outer nuclear layer nor to the rods and cones belonging to them. At the lower irradiation levels the ganglionic layer appeared to be somewhat less susceptible than the inner nuclear layer, but with an irradiation of 60,000 r it, too, was completely destroyed (Fig. 2). The occurrence of structures interpreted as chromosomal vesicles in all

the retinal nuclei is considered to be normal because they are the same in experimental and control animals.

Toads irradiated with 10,000 r or 20,000 r and killed at twenty-four hours (Fig. 4 and Fig. 6) showed considerable destruction of the inner nuclear layer but this was almost complete at 30,000 r and 60,000 r. Toads irradiated at 10,000 r

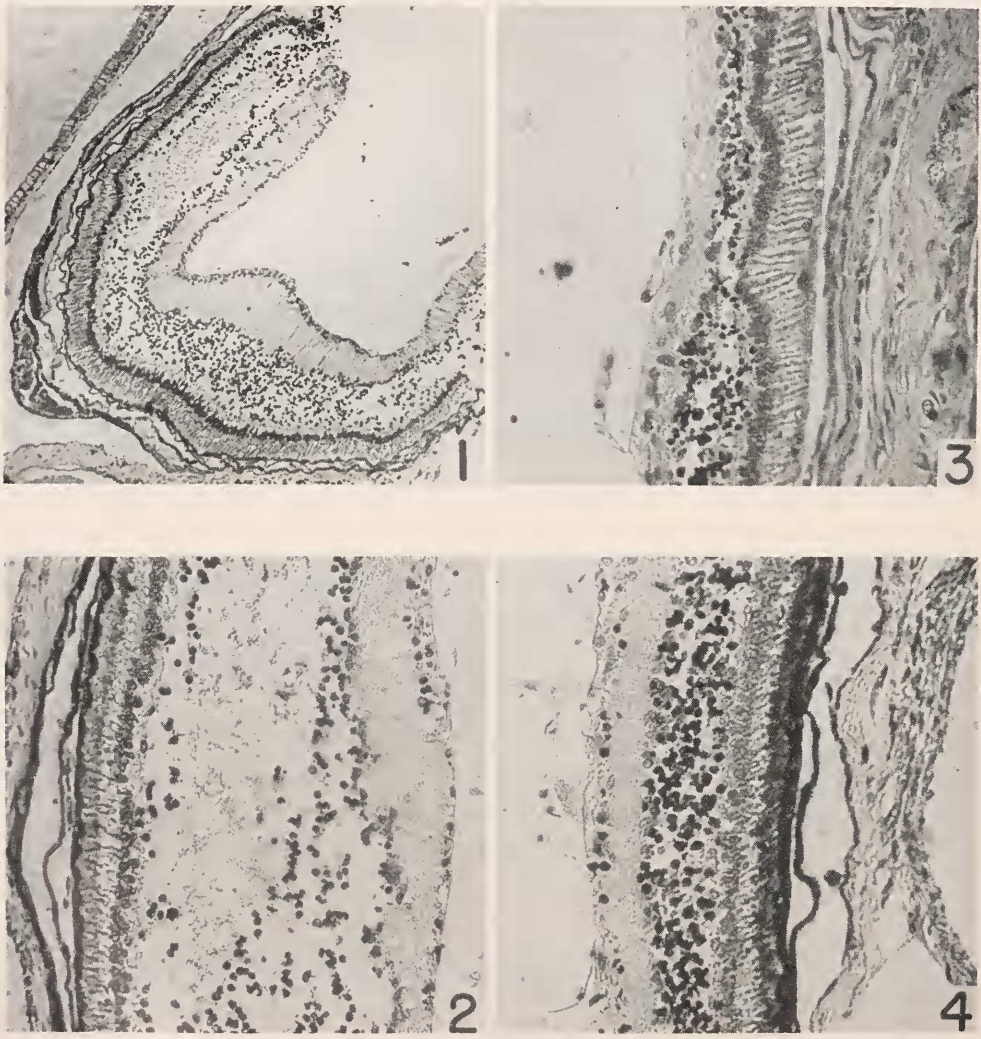

FIGURE 1. The retina at twenty-four hours after being given 60,000 r; × 60.

FIGURE 2. A higher magnification of the area in Figure 1, showing a portion of the more edematous area. × 239.

FIGURE 3. The retina six days after having been given 20,000 r. The thickness of the internal nuclear layer is reduced. The lowest part of the picture is the nearest to the ora serrata. × 329.

FIGURE 4. The retina twenty-four hours after having been given 20,000 r. × 239.

and 20,000 r and killed at an interval of six days after irradiation (Fig. 3 and Fig. 7) showed decided reduction in the thickness of the inner nuclear layer due to resorption of dead nuclei.

With doses of 60,000 r, groups of toads were killed immediately and at short intervals of one hour, two hours, and three hours after irradiation. In general, the effect was a delayed one, pyknosis becoming more and more extensive up to 24 hours. This was observed both after x-ray and gamma irradiation, the results being closely similar. A few cases of pyknosis were seen by the time irradiation was completed. In these early stages pyknosis involved a darkening of the nuclei and only later was there destruction of the cytoplasm. These pyknotic cells occurred throughout the inner nuclear layer of the retina but were most numerous a short distance in from the margin. Not only do the nuclei become deeply pyknotic but decided edema of the retina results. This was localized in the case of lower irradiation dosages, but with 60,000 r the entire retina becomes very heavily pyknotic and edematous (Fig. 1).

Figures 6, 7, 8 and 9 show portions of the retina under different degrees of irradiation. Pyknosis is roughly proportional to the amount of the dose. It is clear that even the highest dose does not affect the outer nuclear layer or the rods and cones, as observed at 24 hours or at six days after irradiation with 20,000 r.

Experiments were performed to show the effect of divided doses on the basis of a 60,000 r total. Irradiation was given on consecutive days and at the following rates: 10,000 r, six times; 20,000 r, three times; and 30,000 r, two times. Destruction of the inner nuclear layer was comparable to that caused by a single dose of 60,000 r. There was a marked reduction in thickness of the inner nuclear layer due to resorption during the course of the experiment (Fig. 8). Divided doses did not cause the amount of edema produced by a single dose.

A very interesting condition is seen at the ora serrata (Fig. 10). The cells of this region show no visible differentiation and there is no sharp line of demarcation between prospective sensory and nerve cell layers, but the nuclei toward the cavity of the eyeball can be followed to the inner nuclear and ganglionic layers, and we consider them to be prospective nerve cell nuclei. It is significant that irradiation renders these highly pyknotic. On the other hand, the nuclei adjacent to the choroid coat, considered to be prospective sensory cells, are not pyknotic. In the region more peripheral to the ora serrata there is likewise no pyknosis in spite of the fact that it is the zone where mitosis had added to the retina, up to a stage shortly before this. It would seem clear that some change has taken place in the prospective nerve cells that renders them especially sensitive to irradiation.

Following the differentiated nerve cell layers central to the ora serrata the distribution of pyknotic nuclei shows that cells of the peripheral portion of the retina are far more readily destroyed than the ones nearer to the center of the retina. At a lower degree of irradiation, 10,000 r and 20,000 r, this is quite evident but at 60,000 r the entire retina is deeply affected.

Discussion

The effects of irradiation upon the retina of amphibians have been chiefly studied in young stages. Brunst (1955) applied x-rays in doses of 1000 r to 8000 r to axolotl larvae from 9 to 65 days of age, the experiment being terminated in 18 to

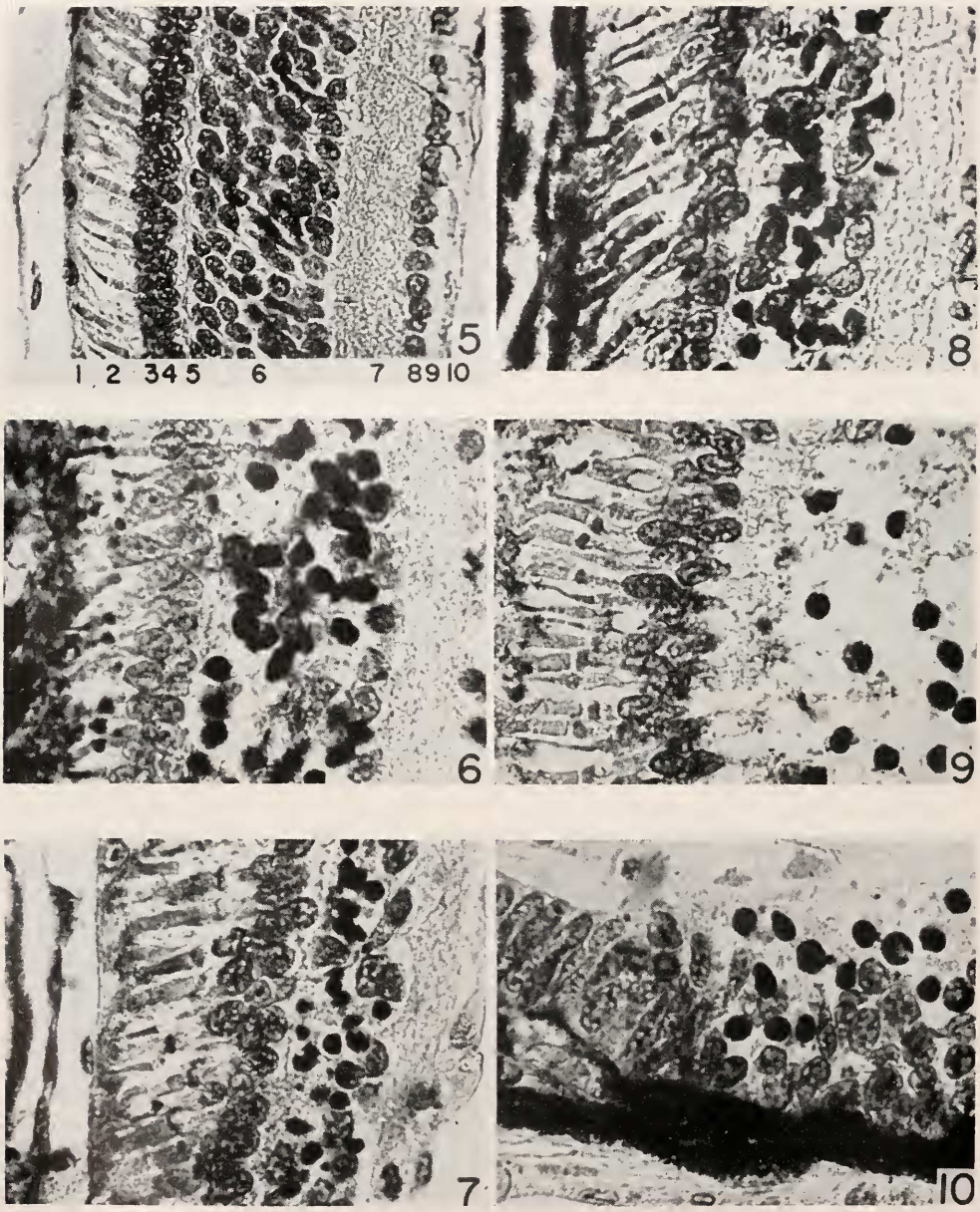

FIGURE 5. Section from the retina of a normal control animal. (1) pigment layer, (2) the rod and cone layer, (3) outer limiting membrance, (4) outer nuclear layer, (5) outer plexiform layer, (6) inner nuclear layer, (7) inner plexiform layer, (8) ganglionic layer, (9) nerve fiber layer, (10) internal limiting membrane. × 1110.

FIGURE 6. The retina twenty-four hours after having been given 20,000 r. × 1402.

FIGURE 7. The retina six days after having been given 20,000 r. Pyknotic nuclei are shrunken and resorbed. The thickness of the inner nuclear layer is reduced as compared to Figure 6. × 1402.

28 days after irradiation. He stated that in all cases the rod and cone layers disappeared first. Eventually the retina degenerated leaving only pigment cells. Brunst (1955) further states (p. 289): "These observations justify the conclusion that the eyes of animals used for this investigation are organs in the process of differentiation and active growth and are therefore, according to the law of Burgonie and Tribondeau, sensitive to roentgen irradiation."

Rugh (1954) finds that in *Ambystoma* larvae 22 mm. in length, x-irradiation of 15,000 r not only causes destruction of mitotic cells, but is equally destructive in regions lying quite apart from them. In a discussion following the reading of this paper, Rugh stated (p. 63), "the rods and cones are separated from the pigment layer but are not individually damaged as are the neuroblast cells. . . . The rods and cones are apparently not relatively sensitive or delicate."

In our own work the rods and cones and the plexiform layers are completely developed (Fig. 5). At the same time this condition has been rather recently attained. The peripheral region of the retina is the youngest part, having been built up as a result of mitotic activity in the region of the ora serrata, as shown by Spear and Glucksman (1941). We have shown that irradiation with 10,000 r and 20,000 r produces very heavy destruction of the inner nuclear and ganglionic layers a short distance central to this region. This leads to the assumption that the susceptibility of these cells is conditioned by their degree of maturity, together with the intensity of the irradiation. In our work we find no evidence that the outer nuclear layer or the rods and cones are affected even by 60,000 r in the 24 hours through which the toads survive. Edema is localized in the case of 10,000 r and 20,000 r irradiation but it is general when 60,000 r is given in a single exposure. It is of secondary importance because it does not appear when 60,000 r is given in divided doses. We have shown that the amount of pyknosis produced by divided doses appears to be roughly equal to that observed when irradiation is given in a single dose. This was shown in *Triturus* by Brunst and Sheremetieva-Brunst (1949).

In sharp contrast to our findings in *Bufo*, the investigators who have irradiated the eyes of mammals have found most extensive injury to the outer nuclear layer, notably to the nuclei of the rods and to the rods themselves. The cones with their nuclei were less affected. This was the finding of Lorenz and Dunn (1950), who exposed newborn mice to 400 r of x-irradiation and killed them at the end of twelve months. Noell *et al.* (1954) gave x-irradiation to the eyes of rabbits, resulting in the destruction of the visual cells while the inner retinal layers were spared. Similar findings were recorded by Brown *et al.* (1955), and Cibis and Brown (1955), who used gamma and x-irradiation upon the monkey, *Macaca rhesus*. With doses of 10,000 r the rods and their nuclei were affected as early as two hours after irradiation. Only when doses exceeded 30,000 r, was there considerable destruction of nerve cells of the inner nuclear layer.

FIGURE 8. This animal was given 10,000 r on 6 consecutive days. The amount of pyknosis is comparable to that produced by 60,000 r in one dose. The thickness of the inner nuclear layer is much reduced by resorption. There is no edema. × 1402.

FIGURE 9. The retina twenty-four hours after having been given 60,000 r. A large amount of edema and many pyknotic nuclei. × 1402.

FIGURE 10. Undifferentiated margin of the retina near the ora serrata, twenty-four hours after 60,000 r was given. The pyknotic nuclei are continuous with the inner nuclear and ganglionic layers. The pigment epithelium is toward the bottom of the picture. × 1402.

Noell (1953, 1955) experimented upon rabbits by injecting sodium iodate, sodium iodacetate, and also by applying oxygen poisoning as well as x-irradiation. All of these were especially injurious to the rods and in a lesser degree to the cones. They all produced quite comparable effects. The nuclei of the outer nuclear layer were largely killed except those belonging to the cones. He found that with the sodium iodate-poisoning, the pigment epithelium was first affected and the injury to the sensory organelles came later, leading to the view that there is a causal relation.

It is clear that in all of the above papers dealing with experiments upon mammals, it was shown that the rods and their cell bodies in the outer nuclear layer were most sensitive to irradiation, while the nerve cells were affected in far less degree. We have shown that just the reverse is true in *Bufo,* within the time limits used. In fact, we find no injury to the rods and cones and their cell bodies in the outer nuclear layer, while on the other hand, the inner nuclear and ganglionic layers show a very high degree of pyknosis. We shall not attempt in this paper to speculate upon the reasons for this difference but the facts are clear enough, and the point is most significant.

Attention is called to the sensitivity of the prospective nerve cells observed at the ora serrata where structural differentiation has not yet taken place. It is evident that in very early stages these prospective nerve cells have already undergone invisible changes that render them vulnerable to irradiation.

Allen (1956) showed that irradiated toads used in this work not only suffered heavy destruction of the nerve cell layers of the retina but also of the nerve cells of the brain.

Summary

1. Gamma irradiation doses of 10,000 r, 20,000 r, 30,000 r and 60,000 r were given to recently metamorphosed *Bufo boreas halophilus.* Toads receiving the two lower dosages were killed at the end of twenty-four hours and at six days. Those receiving the two higher dosages were all killed at twenty-four hours. Cell destruction was found in the inner nuclear and ganglionic layer of the retina but none was found in the outer nuclear layer nor in the rods and cones.

2. Destruction increased as the dosage increased. At doses of 10,000 r and 20,000 r, pyknotic nuclei were most abundant near the marginal portion of the retina, where the cells had been formed more recently than in the central portion. In animals that were given 60,000 r, pyknosis of retinal nerve cells was general and almost complete.

3. Divided daily doses of 10,000 r given six times, 20,000 r three times, and 30,000 r two times produced as much destruction as a single dose of 60,000 r. The amount of edema in these cases was never as great as that which was caused by a single dose of 60,000 r.

4. At the ora serrata where structural differentiation was not yet evident, cells interpreted as prospective nerve cells were destroyed while there was no destruction of prospective sensory cells of the outer nuclear layer.

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